•ACTIVE IMMUNIZATION IN THE PREVENTION OF BIOMATERIAL CENTERED INFECTION

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INTRODUCTION

Total knee arthroplasty (TKA) is a common operation in the United States. A recent study[1] looking at the current mechanisms of total knee replacement failure listed infection as the fourth most common reason for revision surgery. Other series[2-4] have ranked infection as the primary cause of failure, with 64% of patients having their revision surgery within 5 years of their index procedure. Infection has been estimated to occur in as low as 0.39% of primary TKA[5] and 6.7% in revision surgeries[6]. An infection is a devastating complication leading to poor outcome.

Commonly encountered organisms in TKA infection[7] include *Staphylococcus aureus* (50%), coagulase-negative *Staphylococci* species (30%), and *Enterococci* (13%). Over the past 10 years, infections with methicillin-resistant *Staphylococcus aureus* (MRSA) strains are becoming more frequent[8]. In a recent study[9] looking at the results of the surgical treatment of infected TKA, the success rate was 18% for MRSA compared to 89% for the non-resistant strains. The efficacy of a *Staphylococcus*-specific vaccine in the prevention of biomaterial centered infections in an established *in vivo* rabbit knee model[10] was investigated. The vaccine (StaphVax®, Nabi, Rockville, MD) is composed of *Staphylococcus aureus* type 5 and 8 capsular polysaccharides conjugated to an equal weight of recombinant exoprotein A, and has been previously demonstrated to confer protection against *S. aureus* bacteremia in ESRD patients on hemodialysis.[11]

METHODS

Thirty-two skeletally mature New Zealand White rabbits were used in this study. They were allowed to acclimatize for 1 week before the start of the study and their mean weight at that point was 4.2 kg. The Animal Care and Use Committee of the University of Virginia approved all experimental protocols. Twenty-three animals were vaccinated with 50 mcg of StaphVax® (Nabi, Rockville, MD) at day 0, 21 and 42. Nine controls, or non-vaccinated animals, were administered with saline instead of vaccine. Antibody titers were obtained on the days of vaccination and at the time of surgery to determine effectiveness. Animals with low titers on day 42 were boosted on day 49 prior to surgery on day 52 or 54 and if titers were above the 100 mcg/ml threshold (baseline <1mcg/ml), the animals were made immediately available for surgery. After administration of anesthesia, both knees were shaved, prepped with betadine and a 70% ethanol solution, draped, and a lateral arthrotomy was made exposing each lateral femoral condyle. A 3.5mm diameter hole was drilled into the lateral femoral condyle. Doughy polymethylmethacrylate (PMMA) cement (Osteobond, Zimmer Inc., IN) was injected into the defect followed by the insertion of a 15mm long 3.5mm stainless-steel cannulated screw with a high molecular weight polyethylene (UHMWPE) washer. After closure of the joint capsule, one knee was injected with a target inoculum of 10^3 colony-forming units (CFU) of MRSA (STO21) and the other with twice the inoculum in a blinded fashion. Animals were sacrificed after seven days, and a total of five samples (synovial fluid, fascia, necrotic tissue, bone and biomaterials) per knee were examined for evidence and magnitude of bacterial growth. Each knee was visually evaluated and graded for the intensity of the infection: Grade 0 no signs of infection, Grade 1 superficial fibrinous exudate, Grade 2 superficial and deep fibrinous exudate, and Grade 3 gross puss. Bacterial growths from the implant surfaces and surrounding tissues were quantitated. A blood sample, liver and kidney biopsies were also tested for infection. Biomaterial centered infection was defined as bacterial present on the screw/washer in addition to two other tissue sites.

Data was analyzed to evaluate the efficacy of the vaccine in an experimentally created biomaterial centered infection model. Statistical analysis was performed using Statistical Analysis Software (SAS institute). General equation estimation model[12] was used to examine the association between incidence of rabbit knee infection and vaccination status, and the effect of vaccination on individual samples. This animal infection model correlates between both knees from the same rabbit and the actual bacterial inocula. Both logit and probit links were attempted and yielded similar results. Similar analysis was performed using antibody titers to explore their preventive effect. Bacterial inocula and antibody titers were analyzed using logarithm transformation in base 10. Odds ratios (OR) and their 95% confidence intervals (CI) are reported.

RESULTS

Final results are presented with logit link. All animals in the vaccinated group achieved a titer at least 10-fold higher than baseline. Vaccine titers of specific IgG on the day of surgery ranged from 33 to 245 mcg/mL, with a median of 118 mcg/mL, and a mean 124 mcg/mL (SD 59.5). Vaccinated animals demonstrated a decreased incidence of MRSA biomaterial infection compared to controls (OR, 0.24; 95% CI, 0.06-0.96, p=0.0442). The protective effect of vaccination against *S aureus* was detectable in individual tissues when the knee joint was challenged with MRSA (Table 1).

Site	Actual titers scenario			10-fold increase in titers scenario		
	OR	95% CI	p	OR	95% CI	p
Aspirate	0.28	0.07-1.05	0.059	0.52	0.28-0.97	**0.039**
Fascia	0.17	0.02-1.5	0.11	0.41	0.15-1.12	0.081
Debris	0.21	0.05-0.89	**0.035**	0.47	0.25-0.88	**0.019**
Screw	0.24	0.06-0.96	**0.044**	0.51	0.27-0.95	**0.034**
Bone	0.41	0.12-1.42	0.159	0.7	0.41-1.2	0.197

Table 1. Infection OR for 1- & 10-fold *S. aureus* specific IgG titer levels

This protective effect of the animal's response to the vaccine based on preoperative specific serum IgG titers and the incidence of MRSA infection was examined. The incidence of biomaterial-centered MRSA infection in animals with specific serum IgG titers in the upper 50th percentile was less than the incidence of infection of animals in the lower 50th percentile (OR, 0.34; 95% CI, 0.10-1.14, p=0.0798). Evaluating incidence of infection revealed a significant reduction in the incidence MRSA biomaterial infection with a ten-fold increase in the specific IgG serum titers (OR, 0.51; 95% CI, 0.27-0.95, p=0.0345). Overall, when compared to controls, vaccinated animals demonstrated a decreased probability of biomaterial-centered infection with increasing challenge of MRSA (figure 1)

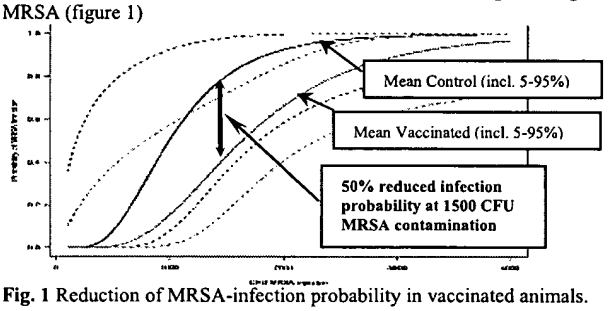

Fig. 1 Reduction of MRSA-infection probability in vaccinated animals.

DISCUSSION

This rabbit knee model utilizes commonly employed orthopaedic implant materials in an *in vivo* milieu and provides an effective method for the evaluation and treatment of biomaterial centered infections. With the growing prevalence of antibiotic resistant organisms, active immunization using the StaphVax® (Nabi, Rockville, MD) is a promising anti-infective strategy. Raising specific IgG titers to battle the most important biofilm forming organisms known to cause biomaterial centered orthopaedic infections is vital in the prevention of costly acute postoperative infections in high-risk patient populations.

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REFERENCES

1) Sharkey PF, et al. Clin Orthop 404; 7-13, 2002. 2) Fehring TK, et al. Clin Orthop 392; 315-8, 2001. 3) Rand JA, et al. Orthop Clin North Am 13; 201-12, 1982. 4) Cameron HU, et al. Clin Orthop 170; 141-6, 1982. 5) Peersman G, et al. Clin Orthop 392; 15-23, 2001. 6) Saleh KJ, et al. J Arthroplasty 17(8); 967-77, 2002.7) Rasul AT Jr, et al. Clin Orthop 273; 98-104, 1991.8) Gristina AG, et al. Science 237; 1588-95, 1987. 9) Kilgus DJ, et al. Clin Orthop 404; 116-124, 2002. 10) Craig MR, et al. abstract, ORS 2004. 11) Shinefield H, et al. N Engl J Med 346; 491-6, 2002. 12) Diggle PJ, et al. Analysis of Longitudinal Data. 2nd ed. New York City, NY. Oxford University Press; chap 8, 2002.